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Resumen por el autor, Frank C. Mann.

Un páncreas accesorio en la pared de la vejiga biliar de un perro.

En un pequeño perro adulto ha encontrado el autor un páncreas accesorio que media 15x10x4 mm., situado en la superficie libre o inferior de la vejiga biliar. El estudio de cortes microscópicos ha demostrado que dicho páncreas consta de acinos normales, aunque bastante pequeños. Después de buscar cuidadosamente, el autor ha encontrado algunos islotes muy pequeños. No existía conexión entre el páncreas accesorio y el páncreas normal, de tal modo que la secreción de la glándula aberrante solo podía verterse en la vejiga biliar.

Translation by José F. Nonidez
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AN ACCESSORY PANCREAS IN THE WALL OF THE GALL BLADDER OF A DOG

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TWO FIGURES

In a previous report I recorded the finding of accessory pancreases in two dogs.¹ I herewith report a third case, which is of interest because of the unusual position of the pancreas, in the wall of the gall bladder. The wide distribution of accessory pancreases along the stomach and small intestine, and the fact that the ventral pancreas normally communicates with the bile duct, lead one to anticipate the possibility of finding occasionally an aberrant gland opening into the gall bladder, but thus far there seems to be no case on record of such an occurrence. Lobes of pancreas extending outward along the bile ducts to the region of the gall bladder have occasionally been reported in the cat, but these are anomalies of quite another sort.

THE FINDING OF THE GLAND AND ITS DESCRIPTION

Dog F 212 (Experiment 216-22). A brown and white female adult mongrel fox terrier. The dog's abdomen was opened, February 22, 1922, in order to ligate the common bile duct for the purpose of studying certain phases of obstructive jaundice. On inspecting the biliary tract, a small mass, noted on the surface of the gall bladder, grossly appeared to be pancreatic tissue. The gall bladder was removed and also a small specimen of the free duodenal portion of the pancreas. The liver and pancreas were normal grossly.

The gall bladder was normal in size, measuring 5 cm. in length and 2.5 cm. in width at the broadest portion. The mass, which

¹ Mann, F. C.: Accessory pancreas in the dog. *Anat. Rec.*, 1920, xix, 263-268.

proved to be an accessory pancreas, was located squarely on the free or inferior surface of the gall bladder, and extended backward from the beginning of the neck. It was roughly elliptical in shape. The long axis measured 1.5 cm. and the short axis 1 cm.; it varied from 2 to 4 mm. in thickness. The surface of the gland on the serosa side was irregular and lobulated like a normal pancreas, except that the lobules were small. The mucous membrane of the gall bladder over the gland was not broken, and did not appear different from any other portion, except that there were three pin-point openings. Under the dissecting microscope these appeared to be the openings of ducts. Unfortunately, in the histological sections which were made, it was impossible to follow any of the ducts through to an orifice in the gall bladder, although these ducts were present near the site of the apparent openings. The blood supply to the gland was generous (fig. 1).

Microscopic examination showed the gland to be composed of pancreatic tissue similar to that of the major pancreas (fig. 2). It was normal, except that the acini seemed to be rather small, and there were no well formed islands. There were a few large ducts and several small ones. The acinar cells were closely packed giving a compact appearance to the gland. Small groups of cells apparently island cells, usually not more than six in number, were found at intervals. The careful study of many slides of three accessory pancreatic glands in the dog demonstrated one characteristic common to all, namely, the very small amount of island tissue. In the present case, after a prolonged search, a few definite but very small islands were found. The condition of the acinar tissue in connection with a well developed duct system definitely indicated an active gland, and it was absolutely isolated in every respect from the major pancreas. We examined very carefully around the bit of pancreatic tissue for some indication of a connection between it and the major pancreas, but none was found. Histological sections through the inferior end of the accessory gland failed to disclose any evidence of such a connection. The larger ducts as before noted were toward the gall bladder, into which undoubtedly the secretion was discharged.

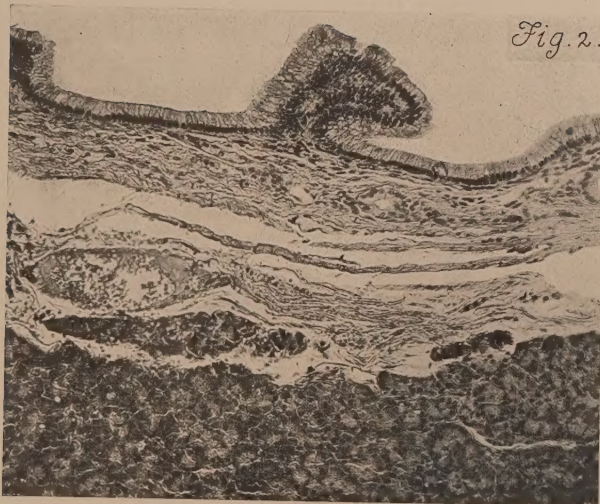
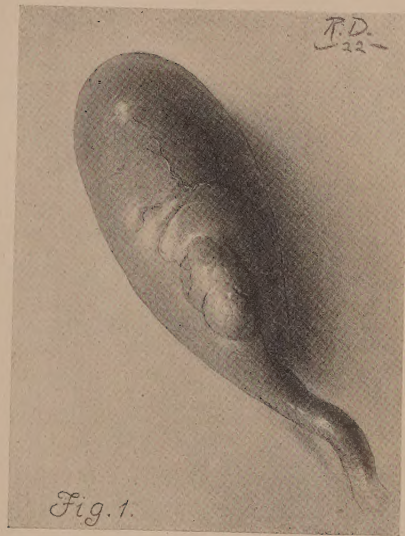


Fig. 1 Drawing of the gall bladder, showing the relative position and size of the accessory pancreas.

Fig. 2 Photomicrograph of section of accessory pancreas and gall-bladder wall. The gland was located just under the serosa. ($\times 100$).

Resumen por el autor, Benjamin T. Nelson.

El número de glomérulos en el riñón del conejo adulto.

El autor ha contado los glomérulos en riñones, en los cuales dichos elementos se habían teñido previamente mediante coloración supravital con verde janus. En uno de ellos ha contado el autor el número total, que resultó ser 163,075. En otros ocho riñones procedentes de cuatro animales, cálculos basados en el peso total de la corteza y en el recuento de un riñón previamente pesado han resultado en cifras que oscilan entre 118,160 y 168,966. Con una sola excepción el número de glomérulos encontrado excede 154,000, y el número medio para todos ellos, incluyendo el riñón contado in toto es 157,180. El número de glomérulos en cada riñón del conejo adulto es por consiguiente unos 160,000, próximamente.

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THE NUMBER OF GLOMERULI IN THE KIDNEY OF THE ADULT RABBIT

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The number of glomeruli in a kidney is a measure of the number of uriniferous tubules, and as such a necessary factor in estimating the relative surface of glomerular and tubular epithelium concerned in the secretion of the urine. It is also an important element in Brodie's computation of the pressure necessary at the glomerular end of the tubule to drive the urine along the tubule at the rate at which it is secreted in diuresis.

The earliest enumeration of the tubules is credited to Eysenhardt (1818), whose article I have, unfortunately, been unable to consult. According to Huschke ('44), Eysenhardt estimated the tubules of the human kidney at 42,000,000—a number which Huschke criticised as probably much too high because of the enumeration of blood vessels as tubules. Through an error Huschke is usually credited by authors as the first to enumerate the glomeruli, but his article in Oken's *Isis*, vol. 21, 1828, to which reference is made by Brodie, Policard, and others, contains no mention of the number, except the remark that they are more numerous, relative to mass, in the young than in the adult. Not until 1844, however, did Huschke discuss the actual number of tubules in the human kidney. In his article entitled "Einge-weidelehre" in Sömmering's *Bau des menschlichen Körpers* he said: "Every kidney lobe contains about 700 kidney lobules and each lobule about 200 cortical canals. If the kidney has 15 lobes that would give it 10,500 lobules and 2,100,000 cortical canals."

In his classical work on the kidney published in 1865, Schweigger-Seidel ('65) describes his method of determining the number of glomeruli in the kidney of the pig and discusses the results.

After carefully separating the cortical substance from the rest of the renal substance, he found, in a kidney weighing 120.5 grams, 102 grams of cortex. Small weighed portions of this cortex were teased and macerated in hydrochloric acid to permit the separation of the glomeruli for counting. In a total weight of 15.5 cgm. he found 720 glomeruli, and calculated the total content of the kidney as 473,200 glomeruli.

Peter's ('09) estimate of the number of tubules in the cat is based on the mode of branching of the ducts. He says: "Bei der Katze ergeben sich stets 4 initiale Aeste und meist 7 zentrale Teilungen; auf einen ins Becken mündenden Gang entfallen damit (2⁸). 4 Kanälchen, d.h. 1024; nehmen wir die Zahl der Sammelröhren 1. Ordnung wie beim Hund auf 200-300 an, so ergäbe dies für jede Niere 200,000-300,000 Harnkanälchen." The number of tubules arising from a duct of the first order, 1024, was incorrectly quoted by Policard ('08) as Peter's estimate for the whole kidney.

Miller and Carlton in 1895 made an enumeration of the glomeruli in the cat kidney, based on a previous determination of the average volume of the cortex. They cut sections in series at a thickness of 0.10 mm. of a kidney injected with Prussian-blue gelatin. The outline of the section traced on paper and measured with a planimeter gave the area. They found that 50 per cent to 83 per cent of the glomeruli represented the actual number, the rest representing duplicate counts of glomeruli appearing in more than one section. They computed the content of an average volume kidney of 12.9 cc. containing 9.03 cc. of cortex in one estimation as from 9,183.49 to 15,325.13 glomeruli. Another estimation gave them from 13,288 to 22,220 glomeruli. The mean of these estimates is 15,664.

Brodie ('14) in 1914, with the assistance of Miss M. G. Thackrah, estimated the glomerular content in two dog kidneys, using a method similar to Miller and Carlton's. Brodie and Thackrah, however, cut a complete series of sections, 8 μ thick, of previously weighed pieces of cortex. In these sections the total number of glomeruli was counted and divided by the number of sections in which on the average a single glomerulus would

appear. In this way a ratio of number of glomeruli to weight was obtained from which the whole content of the cortex could easily be computed. The first dog weighed 11 kgrm., its right kidney weighed 34.5 grams, and the total number of glomeruli was 142,000. A kidney of a second dog, weighing a little over 8 kgrm., contained 125,000 glomeruli.

It is difficult to find a basis for comparison of these results because in the case of the cat only have we estimates made by two investigators. In this case the estimate of Peters is more than tenfold that of Miller and Carlton. The method employed by Miller and Carlton, however, involved the actual count of the glomeruli in series, and thus is less open to objection than that of Peters, who used in his computation a factor obtained from study of the dog's kidney.

If we compare in so far as is practicable the results of Miller and Carlton on the cat with those of Brodie on the dog, we find that Brodie's result, though agreeing fairly well with the estimate of Schweigger-Seidel on the pig, involves a content per milligram of kidney approximately three times as great as that of Miller and Carlton. The recent work of Bremer ('16) on the activity of the mesonephros in the embryos of various mammals, as indicated by the number and size of the glomeruli and by the increase in number and size of them in progressive phases of embryonic development, may throw some light on the variations in glomerular content of the kidneys of different species of mammals. Bremer found "that the different embryos can be classed as those which retain a functional wolffian body until the kidney is ready to take up the work of excretion, and those in which the wolffian body disappears early, before the kidney has developed active glomeruli." He finds also in the placenta of the latter group evidences of anatomical specialization for interchange with the maternal circulation, which justify the assumption of an excretory function on the part of the placenta. Thus it appears probable that the development of the kidney may be influenced by the concurrent existence, functional capacity, and history of other excretory mechanisms. Bremer also points out that within each of the classes which he recog-

nizes, "individual animals are provided with a very varying amount of excreting surface, showing presumably varying types of metabolism." The discussion of these interesting correlations must, however, be postponed, pending a confirmation by newer and better methods of the counts of glomeruli made in other mammals, which must in addition be supplemented by a careful quantitative study of the relative total glomerular surfaces—a study which involves a consideration not only of the number, but also of the size and lobulation of glomeruli.

My determinations have been made by a method essentially similar to that employed by Schweigger-Seidel, except that in most cases I counted the glomeruli in a much larger percentage of the cortex and, in two cases counted the glomeruli in the entire cortex of an adult rabbit's kidney. In addition, the glomeruli were stained so that there was no difficulty in seeing them and distinguishing them from tubules, which might be difficult in the unstained kidney used by Schweigger-Seidel.

The method is essentially the same as that employed by Bensley in the enumeration of the islands of Langerhans in the pancreas. Janus green B when injected by way of the blood vessels in the living kidney, has the property of staining the glomerular tufts intensely, and the stain can be easily fixed permanently by means of ammonium molybdate (Merck or Kahlbaum). According to Cowdry ('18), janus blue has the same property, but we have not employed this dye.

The animal is killed by bleeding from the carotid, the chest rapidly opened and a cannula inserted in the arch of the aorta. Through this 0.85 per cent salt solution is injected until the blood is well washed out of the kidney, when it is followed by a 1 in. 10,000 solution of janus green in salt solution. When the kidney is a uniform blue color, it is covered up by the intestines for fifteen to thirty minutes, to permit reduction of the excess dye. When this process is complete the kidney presents a purplish tint on its surface. Then a 5 per cent solution of ammonium molybdate is injected to check further reduction, and the kidney is removed and placed in a jar containing molybdate. In a successful preparation only the glomeruli will be deeply

stained blue, and as Cowdry (*loc. cit.*) remarked, they can be seen and counted even in thick pieces.

For counting it is necessary to separate the cortex from the medulla, except where a total count is to be made. This involves no great difficulty, since the cortex is deeply stained blue from the numerous glomeruli contained in it. To accomplish this, the kidney is cut into ten or twelve thick slices, out of which the medulla is carefully cut with a scalpel. When this is done a certain amount of fluid escapes, so it is important to determine at the same phase of the work the ratio of weight of sample counted to whole cortex. I have found also that pieces left in ammonium molybdate for some time change considerably in weight. As soon as the cortex is separated from the medulla the total weight is obtained, and a piece selected for counting and immediately weighed.

For counting, the method of teasing and compression is employed. The block to be counted is divided into small fragments which are teased apart and then compressed between two slides, or under a thick cover-glass. Counting is not difficult, and I am confident that the errors of counting are few. Doubtless some error is introduced by incomplete staining and by irregular oedema of the parts of the kidney. Another source of error is incomplete separation of medulla from cortex. As a check on the method of estimation, ten estimates and a complete count were made on the left kidney of a female rabbit weighing 1470 grams. The kidney contained by actual count 227,263 glomeruli. The average of the estimates was 212,269—an error of 6.7 per cent; but the error of the individual estimates varied from 19 per cent plus to 27.7 per cent minus. However, the error of six of the ten estimates was less than 9 per cent and of four less than 4 per cent. A plus error may arise through selecting for counting a piece from the surface of the cortex where the ratio of *pars convoluta* to *pars radiata* is high, and conversely a minus error may arise, as the table shows clearly, from incomplete separation of the medulla from cortex. By care in selection of the sample to be counted, the error may be reduced to less than 10 per cent. The results are contained in the subjoined tables.

The results of these counts and estimates are unexpectedly high. The actual content of glomeruli in a rabbit's kidney is tenfold that admitted for the kidney of the cat by Miller and Carlton, and more than one-third of the content found by Schweigger-Seidel in a pig's kidney fifteen times as heavy. Such differences, if true, suggest interesting physiological and anatomical implications, into the discussion of which, however, we cannot enter at present, pending the confirmation by newer and more accurate methods, of the results obtained by other investigators. This work is now in progress.

TABLE 1

NO.	SEX	BODY WEIGHT	KIDNEY WEIGHT ¹	CORTEX WEIGHT	WEIGHT OF SAMPLE	GLOMERULI	TOTAL
			<i>gm.</i>				
1	M	1990	Total count of one kidney				163075
2	M	1665	R 6.09 L 6.56	3.69 3.73	0.21 0.19	9221 8171	161991 160390
3	F	2145	R 8.40 L 8.68	5.38 5.54	0.36 0.42	10460 11733	156020 154566
4	M	2040	R 8.22 L 8.27	5.4 5.6	0.33 0.175	10326 3704	168966 118160
5	M	2000	R 8.51 L 8.24	5.1 4.6	0.165 0.290	5405 10387	166770 164680

¹ The weights of the kidney given in this table have no value except in relation to the counts, because different kidneys acquire different degrees of oedema in the process of staining.

TABLE 2

Female rabbit, weight 1470 grams

		<i>Glomeruli</i>
Total weight of kidney	9.98 grams	227,263
Total weight of cortex	4.85 grams	223,150
Total weight of medulla	5.13 grams	4,113
(plus cortex in separation)		

TABLE 2—*Continued**Estimations*

NUMBER	WEIGHT	MEDULLA PLUS OR MINUS	COUNT	ESTIMATE	PER CENT OF ERROR
1	0.160	Not observed	7,175	217,488	4.3 minus
2	0.140	Minus	7,843	270,846	19.1 plus
3	0.250	Plus	10,668	206,959	9.3 minus
4	0.280	Plus	9,482	164,228	27.7 minus
5	0.220	Minus	10,597	233,557	2.7 plus
6	0.250	Minus	11,868	230,439	1.3 plus
7	0.250	Minus	11,700	226,980	0.1 minus
8	0.290	Plus	12,395	207,244	8.8 minus
9	0.270	Plus	9,633	173,008	23.8 minus
10	0.230	Plus	9,081	191,427	15.7 minus
Average of the estimates.....				212,269	6.7 minus

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Resumen por la autora, Mary T. Harman.

Sobre el origen del notocordio en el pollo.

Dos pollos anormales producidos en nuestro laboratorio presentaban notocordios excepcionales. Ambos fueron incubados durante tres días. Uno de ellos fué incubado a una temperatura de 106°F. y el otro a una temperatura de 99° a 100°F. En el primer pollo el notocordio es bifurcado en su extremo anterior, en próximamente un tercio de su longitud. En esta misma región el tubo neural está también bifurcado ventralmente. El intestino no presenta indicio alguno de bifurcación. En el segundo pollo el notocordio en el extremo posterior no solo está en contacto con el tubo neural, sino que se continúa con él. El endodermo del saco vitelino está por completo separado del notocordio. Las condiciones excepcionales descritas constituyen pruebas en favor de la teoría del origen ectodérmico del notocordio.

Translation by José F. Nonidez
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CONCERNING THE ORIGIN OF THE NOTOCHORD IN THE CHICK¹

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ONE PLATE (FIVE FIGURES)

The presence of the chorda dorsalis or notochord sometime during the life-history is one of the characteristics which distinguishes the Chordata from the other phyla of the animal kingdom. As to its origin, few other embryonic structures have received more attention from investigators, and yet there exists the greatest diversity of opinion concerning its early embryonic development. All three germ layers have been given as its origin. As early as 1881 Hatschek described the notochord as being folded off from the primitive gut. This is the view held by Hertwig ('05), McMurrich ('15), and others. Prentiss and Arey ('17) say that "the head (notochordal) process and mesoderm of higher vertebrates are not clearly of entodermal origin, but are derived from the ectoderm, any union with the entoderm being secondary." Huber ('18) also considers the notochord to be of ectodermal origin, which becomes associated with the entoderm, and then afterward separated from it. King ('03) says that in *Bufo lentiginosus* the anterior part of the notochord is entirely mesodermal in origin, and that in the posterior part it is mostly derived from mesoderm, but that there is added to it a layer of entoderm from the archenteron.

These differences of opinion are not entirely due to the fact that the investigators have been working with different vertebrates, for Foster and Balfour ('83) say that in the chick the notochord in most instances arises from the anterior end of the

¹ Contribution from the Zoological Laboratory, Kansas State Agricultural College, no. 57.

primitive streak and remains attached to the 'hypoblast' (entoderm), but in other cases the notochord appears to become differentiated in the already separated layer of mesoderm. Pren-tiss and Arey ('17) say that the notochordal plate in birds is mesodermal in origin and later becomes fused with the entoderm. However, they state that the mesodermal plate which goes to form the notochord arises from the thickened ectoderm of the primitive streak.

These great differences of the opinions of able investigators have led the writer to record some abnormal conditions in the notochord of some teratological specimens of the chick produced in our laboratory (Harman, '18; Alsop, '19). It is not the purpose of the writer to give an extended discussion of the vast amount of literature on the origin of the notochord which must of necessity include the origin of the germ layers, but rather to present the conditions as found in these specimens and discuss to some extent the possible bearing upon the theories of the origin of the notochord.

The illustrations are taken from two of the specimens referred to above. Both chicks were incubated for three days. One was incubated at a temperature of about 106°F., and in addition to a very abnormal central nervous system has a bifurcate notochord. The double condition is in the anterior end and extends caudad to the region of the origin of the vitelline veins. Transverse sections of this specimen are represented in figures 1, 2, and 3. The other chick was incubated at a temperature of between 99°F. and 100°F. The abnormal condition of the notochord is shown in figures 4 and 5.

Figure 1 is a section through the chick about 50 μ from the extreme anterior end of the notochord. The notochord, marked *nc* in the figure, is rather small, cylindrical in shape, and instead of being in a median line ventral to the hind-brain, marked *nt* in the drawing, it is in two parts or cylinders located a little to either side of the midventral line. The hind-brain in this region encloses two cavities, a larger dorsal cavity and a very small ventral one. The small cavity is slightly forked ventrally. The respective horns are in a direct line with the noto-

chord of that side. The pharynx, marked *g* in this section, has no indication of the double condition dorsally.

The notochord is double for a distance of about $1\frac{1}{4}$ mm. or through the heart region, a distance of about one-third of its entire length. Figure 3 is a drawing of a section at the point of the convergence of the two horns of the notochord. Figure 2 is a drawing of a section about midway between the sections represented in figures 1 and 3. It will be noted that in figure 3 the notochord is large compared with the double notochord represented in the other two figures. It also has a slight indication of its double condition. The neural tube here also is somewhat double ventrally. Posterior to this region, both the notochord and neural tube are single and appear to be normal for this stage of development. Anterior to this region, the two parts of the notochord gradually diverge and the respective horns become smaller to the region represented in figure 2. Then they come a little closer together, but remain about the same size, as is shown in figure 1. The two cavities of the central nervous system are found only in three sections, but the bifurcate condition of the ventral side of the central nervous system is very distinct in the entire region of the double notochord.

Figures 4 and 5 are drawings of sections through the posterior third of a three-day chick incubated at a temperature between 99°F. and 100°F. The section represented by figure 4 is about $\frac{1}{3}$ mm. anterior to the other one. The neural tube in this region is not quite normal, but its abnormality is more striking a few sections anterior where they are two and three central canals as previously described (Harman, '18; Alsop, '19). As shown in figure 4, the notochord seems to be directly continuous with the neural tube, although the two are almost separated. The entoderm, *en*, of the yolk sac lies close to but entirely separated from the notochord. Posteriorly, as shown in figure 5, the continuity of the notochord with the neural tube is more conspicuous. In fact, the constriction between the notochord and the neural tube is not very deep, and an examination of the cells with the high power of the microscope shows that they are of the same character in both of the structures. The entoderm of the yolk sac is in contact with the notochord, but is distinct from it.

In the case of the first chick, two hypotheses may be considered: either the notochord became bifurcate at the time of its formation or after it was formed. If the former, the factor or factors which by their influence caused the doubling would have affected the structure from which the notochord originated and we should expect an indication, at least, of a double condition of this structure; if the latter, we must suppose the doubling to be accompanied by a growth in its diameter, since each of the paired notochords is of nearly normal size. It is well known that increase in diameter is not normally noticeable in the chick notochord, as the chief difference between that of a one-day chick and that of a three-day chick is in its length. It is evident that increase in length could not produce bifurcation. Therefore, it is hardly probable that bifurcation occurred after the notochord was formed, and we can return to the assumption that the notochord became bifurcate at the time of its formation.

Surrounding the notochord is mesoderm, ventral to it is the gut which is entodermal in origin, and dorsal to it is the neural tube which is ectodermal in origin. If the notochord came from the surrounding mesoderm, it probably developed in situ, since there is no indication of a doubling of the mesodermal structures. The writer sees no condition in favor of this hypothesis and on the other hand no direct evidence against it. If it has arisen in situ it is unique among compact structures of embryonic development which usually arise from a region of unequal proliferation of cells which finally results in a folding or in the formation of a thickened plate. Since there is no evidence of an unequal proliferation of mesodermal cells, the indirect evidence is rather against the theory of the mesodermal origin of the notochord.

If we assume that the notochord is entodermal in origin, we will have to accept that it came from the gut or from the yolk sac before the gut was formed, for these are the only structures of this region of entodermal origin. There is no indication of a doubling of the gut; therefore, from our hypothesis the notochord cannot be entodermal in origin. Then it must be ectodermal, since, as we have shown, it is hardly probable that it is either mesodermal or endodermal. Let us examine the evidence for the hypothesis of its ectodermal origin.

If it were ectodermal in origin, it must have come from the ventral surface of the neural tube. As we have shown previously, the neural tube in the region of the double notochord is double ventrally and where it is single the neural tube is also single. Furthermore, in a normal chick the notochord lies ventral to the median line of the neural tube and the single *canalis centralis* is in direct line with the notochord. In this specimen the *canalis centralis* is forked ventrally. If the respective forks of the *canalis centralis* should be considered as being in the ventral portion of a complete neural tube, then the notochord of the respective sides would have a normal position with reference to the neural tube.

It would seem, then, either that some factor or factors affected the notochord and the neural tube, at the same time causing both of them to bifurcate at least partially, or that they caused the formation of two neural grooves instead of one and that the notochord arose from the ventral surface of the neural tube. This seems all the more probable since both structures are double in the same place and since they are the only structures of this region showing this condition. Therefore, the facts indicate that the notochord became forked at the time it was formed and that it came from the ectoderm.

The condition in the second chick gives added evidence that the notochord has arisen from the ventral part of the neural tube or at least from the ectoderm from which the neural tube is formed. As was given above in figure 4, the neural tube is continuous with the notochord, while in figure 5 not only are they continuous, but it is impossible to tell where neural tube stops and notochord begins. Moreover, the entoderm of the yolk sac is completely separated from the notochord. It seems, then, that the notochord is ectodermal in origin.

To summarize, then, the bifurcate condition of the notochord and a similar condition in the ventral portion of the neural tube in the case of the first described, together with the absence of evidence of a double condition in any other structure in this region, is evidence in favor of the theory that the notochord in the chick is ectodermal in origin. In the case of the second chick, the continuity of the cells of the neural tube with those of the

notochord as well as the complete separation of the entoderm of the yolk sac from the notochord is added evidence for the theory of the ectodermal origin of the notochord.

✱

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PLATE 1

EXPLANATION OF FIGURES

All figures were enlarged about 135 diameters and then reduced one-half.

Figures 1, 2, and 3 are sections through a three-day chick incubated at 106° F. Figures 4 and 5 are sections through a three-day chick incubated at 99° F. to 100° F.

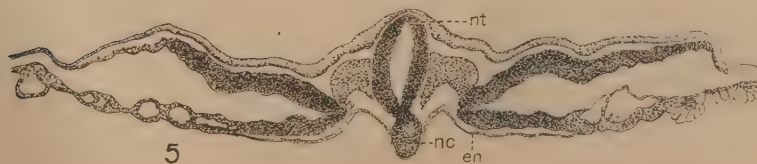
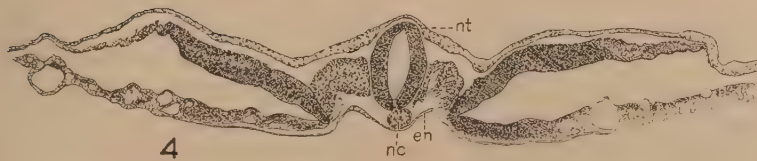
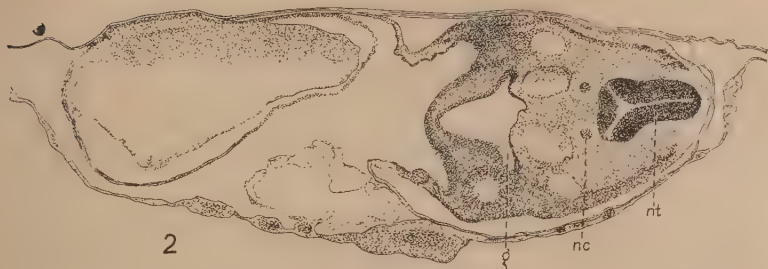
1 Section through the anterior portion of the notochord. *nc*, notochord; *g*, gut; *nt*, neural tube.

2 Section through the middle of the heart. *nc*, notochord; *g*, gut; *nt*, neural tube.

3 Section through the origin of the vitelline veins. *nc*, notochord; *g*, gut; *nt*, neural tube.

4 Section through the chick about one-third of its length from the posterior end. *nt*, neural tube; *nc*, notochord, *en*, entoderm of yolk sac.

5 Section through the chick about $\frac{1}{2}$ mm. posterior to the section represented in figure 4.



Resumen por el autor, Walter Hughson.

La estimulación eléctrica de los nervios cutáneos. Un método de enseñanza.

La estimulación eléctrica de los troncos nerviosos cutáneos ha sido empleada por el autor como un método de enseñanza de los estudiantes de Anatomía macroscópica. Trotter y Davies han demostrado la exactitud de este método y su uso ha sido valioso por suministrar al estudiante conocimientos prácticos de la distribución final y variaciones de estos nervios.

Translation by José F. Nonidez
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ELECTRICAL STIMULATION OF CUTANEOUS NERVES: A TEACHING METHOD

WALTER HUGHSON

Anatomical Department of the Johns Hopkins University

TWO FIGURES

The usual method of teaching the distribution of cutaneous nerves to students in gross anatomy must be regarded in a general way as unsatisfactory. Careful dissections of individual nerve trunks do little more than indicate roughly the region supplied, while text-books with their variegated illustrations leave in the mind of the student a confused idea of some association between cutaneous sensibility and one or more of the different colors used. Such an interesting and important detail as variation in the ultimate distribution of a given nerve is almost impossible of demonstration with our present methods. In short, the student's whole conception is so vague that after having completed his course in anatomy he promptly loses any detailed knowledge he may have gained by his study of text-books and dissections.

Trotter and Davies,¹ in their work on the innervation of the skin, attempted to establish definite anatomic relations for several cutaneous nerves. Variations in the course of the nerves were so great, however, that this was found impossible. Exposure of the nerve trunk for section which formed part of their experiment often involved a skin incision out of all proportion to the size of the procedure. They therefore devised a method of localization which consisted simply of stimulating the nerve-trunk through the skin with a weak faradic current by a finely pointed unipolar electrode. The sensation of tingling corresponded exactly to the peripheral distribution of the nerve.

¹ Trotter, W., and Davies, H. M., Jour. of Phys., 1909, vol. 38, p. 134.

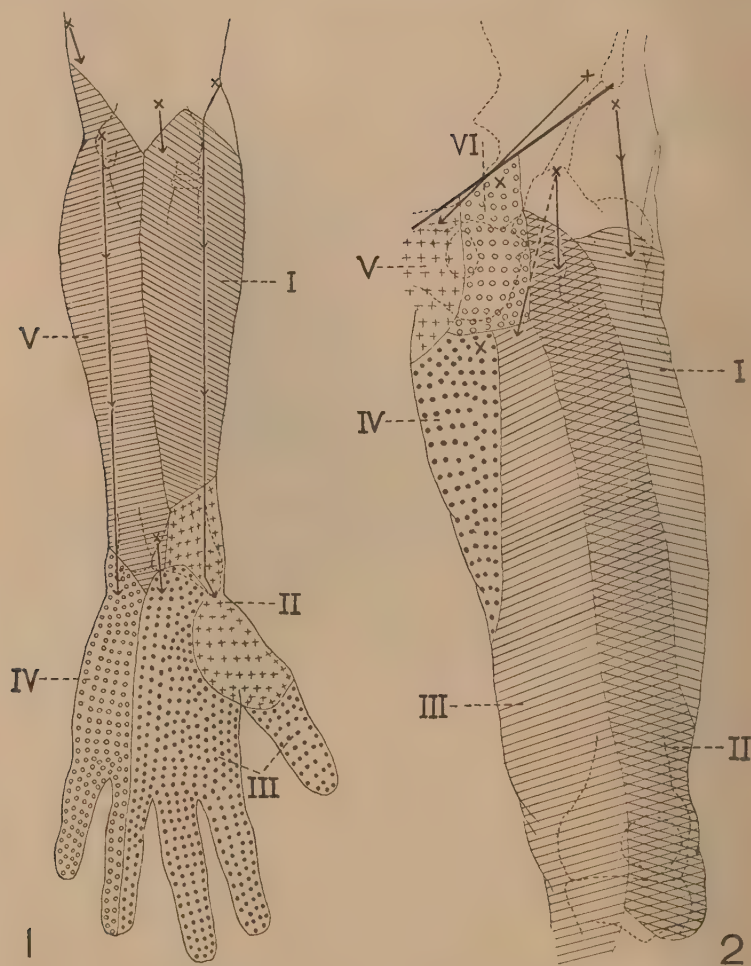


Fig. 1 Volar surface left forearm. *I*, Lateral antibrachial cutaneous; *II*, cutaneous distribution of radial; *III*, of median, and *IV*, of ulnar; *V*, median antibrachial cutaneous.

Fig. 2 Anterior aspect of left thigh. *I*, Lateral femoral cutaneous; *II*, intermediate; and *III*, medial cutaneous branches of femoral; *IV*, cutaneous branch of obturator; *V*, of ilio-inguinal, and *VI*, of genito-femoral.

Application of the stigmatic electrode a millimeter or more to either side of the nerve-trunk failed to elicit any sensation; the accuracy of the method was further demonstrated at operation by finding the nerve immediately beneath the point of localization on the skin. After section, the area of anesthesia corresponded exactly to the distribution determined by stimulation.

The method was used in an elective course in applied anatomy to show the distribution of the cutaneous nerves throughout practically the entire body. A small induction coil (Harvard Instrument Company) was used with a single dry cell. A current considerably weaker than that necessary to cause muscular contraction was found to be sufficient to stimulate the sensory nerves. The indifferent electrode was placed at any convenient point on the body and the stimulating electrode applied to the skin to locate the nerve trunk as it emerged from the deep fascia. The general position of the nerve had previously been determined by the student by dissection. The distinctness of the response to stimulation proved to be very striking and the areas could be mapped out after a small amount of practice with great accuracy.

Figures 1 and 2 represent the type of diagram which the individual student can make. The entire procedure can be carried on without assistance. The nerve is first picked up at the point where it pierces the fascia and can then be followed in all its branches. In the figures, X marks the point of stimulation and the arrow the area of distribution indicated by shading. In figure 1 the arrow shows that stimulation of the radial and ulnar cutaneous nerves along their entire course from the elbow will give the typical sensory distribution. The cutaneous branches of the median nerve, however, can be stimulated on the volar surface of the forearm only at the wrist. The lateral and medial cutaneous nerves of the forearm can best be stimulated above the elbow. Figure 2 represents a fairly typical diagram of the cutaneous innervation of the front of the thigh. The intermediate branch of the femoral becomes superficial higher up than the medial branch, as is indicated by dotted lines, but the whole distribution is clearly shown. The cutaneous

branch of the obturator is surprisingly accessible. Of some interest is the fact that in this individual the obturator of the opposite side had an area of distribution considerably below the knee. The ilio-inguinal can be found very readily above the inguinal ligament. The whole body with the possible exception of the intercostal nerves can be charted in exactly the same manner. Stimulation of the cutaneous branches of the trigeminal, the branches of the cervical plexus, etc., all give the sharp outline indicated in the figures.

This method then gives the student an actual physiological proof of the distribution of his own cutaneous nerves and fixes this distribution firmly in his mind as dissections and pictures can never do. By comparison with other charts he learns that there is a great variation between different individuals and even between opposite sides of the same individual. And finally the simplicity of the apparatus and method makes it entirely practical for use in large classes.

Resumen por el autor, I. M. Thompson.

Anomalía del nervio medio y del músculo flexor sublime de los dedos.

El autor describe una anomalía del nervio medio del antebrazo, el cual estaba enterrado en el vientre radial del flexor sublime de los dedos, en la mayor parte de su curso en el antebrazo. Dicho músculo era también anormal, presentando separados los orígenes radial y húmero-ulnar, y este último estaba subdividido de nuevo inmediatamente debajo del codo en un vientre lateral pequeño y un vientre medio grande. La disposición de los tendones de este músculo en la muñeca era también excepcional. El autor presenta una explicación embriológica de la anomalía descrita, indicando también su importancia práctica.

Translation by José F. Nonidez
Cornell Medical College, New York

ANOMALY OF MEDIAN NERVE AND FLEXOR DIGITORUM SUBLIMIS MUSCLE

I. MACLAREN THOMPSON

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ONE FIGURE

The following anomaly was observed in the dissecting-room at McGill University during the session of 1921-22. As I have not succeeded in finding a description of the condition in the usual records, it would appear to be uncommon, and its importance from a practical point of view makes it the more worthy of being recorded.

The subject was a female, aged eighty-six, with attenuated musculature. The median nerve of the right-arm descended into the forearm between the two heads of the pronator teres in the usual manner. Then it passed deep to the radial origin of the flexor digitorum sublimis, the latter consisting of a slender belly which was not united to the humero-ulnar origin. About 35 mm. distal to this point (i.e., about 130 mm. distal to the medial epicondyle of the humerus) the nerve sank into the substance of the radial belly, and remained completely embedded therein down to a point about 25 mm. above the wrist-joint. Here the tendon of this belly divided into two, a medial and a lateral; and the nerve appeared between them, lying anterior to the lateral tendon and between the medial tendon and that of the flexor carpi radialis. The subsequent course and distribution of the nerve presented no unusual feature.

The following associated conditions may be mentioned. As indicated, the flexor digitorum sublimis was abnormal. In the first place, the radial and humero-ulnar origins remained entirely separate. Just below the elbow the humero-ulnar origin divided into a large medial belly and a smaller lateral belly.

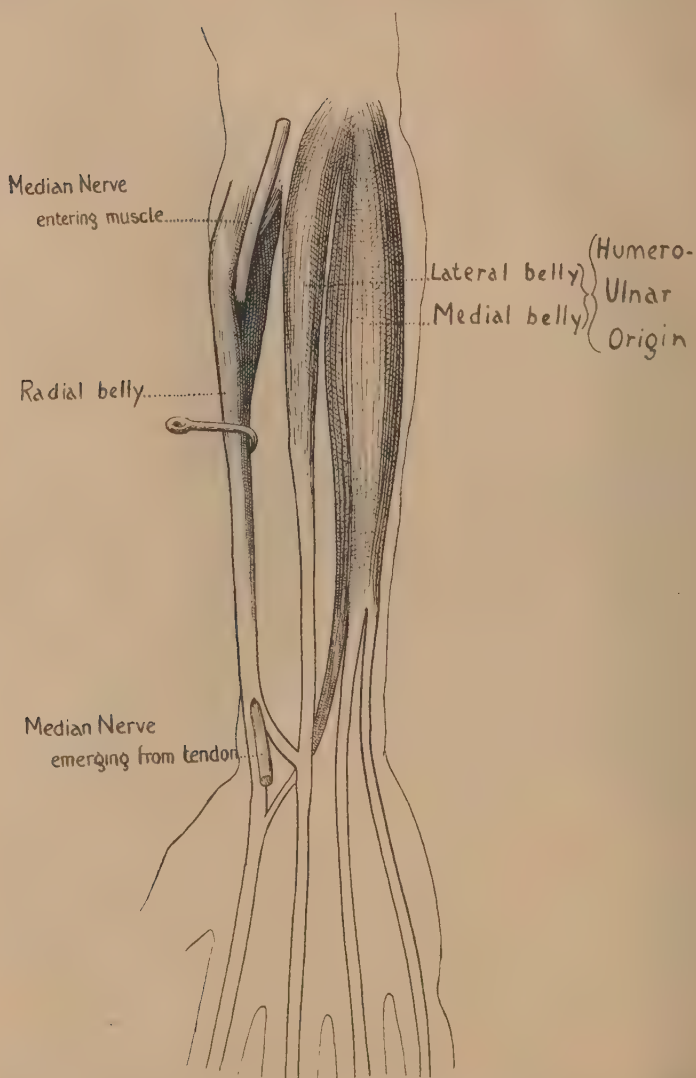


Fig. 1 Right forearm and hand, volar aspect, showing anomalous median nerve and flexor digitorum sublimis muscle. The upper part of the radial belly has been retracted laterally and twisted, exposing its deep or dorsal aspect, with the median nerve entering the muscle. For further description see text.

About 60 mm. above the wrist-joint the medial belly was continued into two tendons, a medial and a lateral, with the addition of a small fleshy lateral slip. The smaller medial tendon went to the little finger, whilst the larger lateral tendon terminated in the ring-finger. About the middle of the forearm the lateral humero-ulnar belly was continued into a tendon which went to the middle finger, being joined at the level of the wrist-joint by the medial tendon of the radial belly.

The slender separate radial belly, in which the median nerve was embedded, arose by fleshy fibers from the lower end of the oblique line of the radius. About the middle of the forearm it gave rise to two tendons, a medial and a lateral, which were closely bound together (enclosing between them the median nerve) down to a point about 25 mm. above the wrist-joint, where they separated. The medial tendon joined the tendon of the lateral humero-ulnar belly to form the tendon of the middle finger. The lateral tendon went to the index finger, being joined about 20 mm. below the wrist-joint by the tendon of the small fleshy slip noted above as arising from the lower end of the lateral aspect of the medial humero-ulnar belly, this fleshy slip having become tendinous where it crossed deep to the tendon of the middle finger, at the level of the wrist-joint. The ultimate insertions of these tendons in the fingers presented no unusual feature.

The palmaris longus was absent on both sides.

On the right side the ulnar artery arose as a collateral branch of the brachial artery opposite the upper end of the insertion of the coracobrachialis. It was small, and passed superficial to the common flexor group of muscles. The radial artery was the direct continuation of the brachial artery, was larger than usual, and gave off the common interosseous artery.

On the left side there were no anomalies except the absence of the palmaris longus.

Normally, the median nerve is closely adherent to the deep surface of the flexor digitorum sublimis, to which it is bound by relatively dense connective tissue. This fact is not stated in the majority of the text-books of anatomy, but is easily

verified by dissection. The present anomaly appears to be a further stage of this normal condition, the nerve not merely adhering to the deep surface of the muscle, but actually being embedded within its substance.

W. H. Lewis (The development of the arm in man; *Am. Jour. Anat.*, vol. 1, 1902, p. 145) describes the median nerve in a human embryo of 9 mm. as extending to the distal end of the humerus, while the distal part of the arm is filled with primitive mesenchymal tissue which later differentiates into muscle and intermuscular tissue.

In an embryo of 10.5 mm. the same investigator describes the flexor muscle mass of the forearm as consisting of a superficial layer and a deep layer, separable with considerable difficulty, the median nerve lying between them.

In the 9-mm. embryo the median nerve has reached the distal end of the humerus, while the premuscle tissue of the upper arm shows no sign of differentiation. Assuming that what occurs in the arm occurs also in the forearm, we may therefore conclude that between these two stages described by Lewis the median nerve grows down into the undifferentiated mesenchyma of the forearm, which thereafter develops two planes of histogenic demarkation, a superficial plane, anterior to the median nerve, along which the superficial muscle layer is differentiated from the layer of connective tissue which intervenes between the superficial and the deep muscles, and a deep plane, posterior to the median nerve, along which the deep muscle layer is differentiated from the same layer of connective tissue. The median nerve thus comes to lie within the layer of connective tissue, between the two planes of demarkation, but nearer the superficial plane, in which region the connective tissue increases in density, thus binding the nerve to the deep surface of the muscle.

The present anomaly has probably been occasioned by the superficial plane of demarkation passing posterior to the median nerve, which was thus included within the superficial layer of muscle tissue from which the flexor digitorum sublimis was later differentiated.

This anomaly would appear to be of some clinical importance, though no information is available in the present case as to the production of symptoms by such an anatomical condition. In this connection it may be noted that normally certain nerves lie embedded in muscle for a portion of their course, as, for example, the deep branch of the radial nerve, which lies embedded in the supinator.

In those cases which call for operative treatment of the flexor tendons or of the median nerve in the forearm, and in which difficulty is experienced in locating the nerve or its ends, if it has been severed, the possibility of the presence of such an anomaly as that described would appear to be worth bearing in mind.

In the present instance, the nerve, as noted, entered the muscle about 130 mm. distal to the medial epicondyle of the humerus, and emerged from the tendon about 25 mm., or one inch, above the wrist-joint. In the living this would correspond approximately to a point about 25 mm., or one inch, above the proximal skin crease at the wrist.

Resumen por el autor, William H. T. Baillie.

Un caso de ausencia unilateral del riñón, uréter y parte distal del tubo uterino del conejo.

El autor describe una caso, hallado en una coneja, de ausencia del riñón y uréter izquierdos, parte del tubo uterino izquierdo y útero del mismo lado. Teniendo en cuenta las condiciones halladas durante el desarrollo embrionario el origen de la anomalía debe haber tenido lugar próximamente en un periodo entre los 11 a los 15 dias del crecimiento embrionario. La relación de este caso con otros publicados es objeto de discusión.

Translation by José F. Nonidez
Cornell Medical College, New York

CASE OF UNILATERAL ABSENCE OF KIDNEY, URETER AND DISTAL PART OF UTERINE TUBE IN THE RABBIT

W. H. T. BAILLIE

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ONE FIGURE

DESCRIPTION

The specimen under consideration is a female with the right side of the urinogenital system normal, save that the right kidney is larger than is usually the case in an animal of the same size. The description of the urinogenital system is as follows:

Left kidney absent; no trace of left ureter; that there was no opening into the bladder on the left side was determined by injecting a semifluid colored mass into the bladder. The right ureter was partially filled.

Left suprarenal body present in normal position, but rotated on its axis through 90° ; supplied with blood from the supracrenolumbar artery.

Left vesicorectal fold present, but contains only the left umbilical artery and does not connect with the vagina.

Right kidney measured 3.4 cm. by 2.2. cm. by 0.7 cm., slightly larger than in normal rabbits of the same occiput-to-tail measurement.

Right ureter normal in position; opening into right side of base of bladder along vesico-uterine fold.

Right suprarenal body normal.

Left ovary slightly longer than right; normal in position. Fimbria, supported by mesovarium, overhangs ovary anteriorly. Oviduct leads to a point near the posterior pole of ovary and ends blindly in a small knob. A narrow mesenterial fold binds oviduct to ovary.

Left ovarian ligament passes from posterior pole of ovary to the point usually reached by left round ligament.

Vagina lies completely to the right. No trace of left uterus.

Left ovarian artery normal in relation. Left ovarian vein passes caudad to join the inferior vena cava just past the point where median sacral artery arises from aorta.

Right portion of genital system normal.

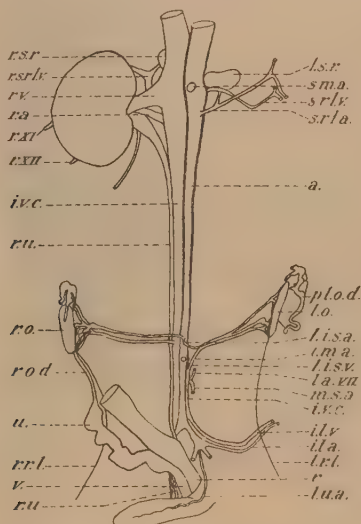


Figure 1. $\times \frac{1}{2}$ natural size

ABBREVIATIONS

<i>a.</i> , aorta	<i>r.a.</i> , renal artery
<i>i.l.a.</i> , ilio-lumbar artery	<i>r.o.</i> , right ovary
<i>i.l.v.</i> , ilio-lumbar vein	<i>r.o.d.</i> , right oviduct
<i>i.m.a.</i> , inferior mesenteric artery	<i>r.r.l.</i> , right round ligament
<i>i.v.c.</i> , inferior vena cava	<i>r.s.r.</i> , right suprarenal body
<i>l.a.VII</i> , seventh lumbar artery	<i>r.s.r.l.v.</i> , right suprarenolumbar vein
<i>l.i.s.a.</i> , left ovarian artery	<i>r.u.</i> , right ureter
<i>l.i.s.v.</i> , left ovarian vein	<i>r.v.</i> , renal vein
<i>l.o.</i> , left ovary	<i>r.XI</i> , eleventh rib
<i>l.r.l.</i> , left round ligament	<i>r.XII</i> , twelfth rib
<i>l.s.r.</i> , left suprarenal body	<i>s.m.a.</i> , superior mesenteric artery
<i>l.u.a.</i> , left umbilical artery	<i>s.r.l.a.</i> , suprarenolumbar artery
<i>m.s.a.</i> , median sacral artery	<i>s.r.l.v.</i> , suprarenolumbar vein
<i>p.l.o.d.</i> , proximal part of left oviduct	<i>u.</i> , right uterus
<i>r.</i> , rectum	<i>v.</i> , vagina

PROBABLE ORIGIN OF THE ANOMALY

In studying the condition it occurred to the writer that it might be possible to determine approximately the age of the embryo when the defect arose. The association of the presence of a perfect ovary and a normal proximal part of the uterine tube with absence of the distal part of the same tube and total absence of the kidney and ureter on the same side would give such a clue. The anterior growth of the ureter and the posterior growth of the müllerian duct have both suffered interference.

According to Minot ('05), the ureter first appears at eleven and one-half days in a 6-mm. embryo rabbit, and at fifteen days in a 12- to 13-mm. embryo the müllerian ducts are present, lying on the coelomic side of the wolffian ducts, extending only a short distance and opening into the coelom at the cephalic part of the wolffian body. In man, according to Felix in Keibel and Mall ('12) "At the very time when the posterior end of the groove is separating from the epithelium it begins to grow out caudally and in this process we come to the development of the distal portion of the müllerian duct. It is formed by the gradual outgrowth of the tip of the cornet." Some interference with growth starting near the caudal end of the mesonephric duct before eleven and one-half days and reaching near the anterior end of the mesonephros at about fifteen days must have been responsible for the condition. If this process is in the nature of an extension of the natural degeneration of the mesonephros the latter must have been markedly premature as well as extensive. This is shown by the normal time of onset of the degeneration. Thus Felix and Bühler, in Hertwig's Handbook ('04), put the degeneration in an 18- to 20-mm. embryo at eighteen days. Bremer ('16) states that the placenta takes over the function of the mesonephros at an age corresponding to 21 mm., when the mesonephros begins to degenerate and ultimately loses all active glomeruli at 40 mm. The tubular degeneration is still later. Thus Felix ('04) says, "Beim Kaninchen beginnt die Rückbildung des primären Harnleiters bei 5 cm. langen Embryonen und zwar in der Mitte und schreitet von da rasch in kranialer und kaudaler Richtung vor und führt zum vollständigen

Schwund des primären Harnleiters (Langenbacher, '82).'' The method of degeneration is, however, similar to that which I have visualized in this case.

COMPARISON

Now, if we compare a few cases of a somewhat similar nature, Hunt ('18) describes the absence of the right kidney in a male domestic cat. The right ureter was present, 1.5 cm. long. The genital system and suprarenals were normal. Since the ureter appears in this specimen, the process has not occurred early enough to cause the inhibition of the evagination. The mesonephric duct is also retained normally as the genital duct. Therefore, the destructive agent acted on the nephrogenic mass shortly after the ureter began its growth and did not involve the mesonephric ducts, but only the part of the nephric mass that normally forms the mesonephric and metanephric glomeruli. The next case is described by Lyon ('17) in man. The right kidney, ovary, and uterine tube were absent. The right suprarenal was present, as was also the right ureter, which was connected with the bladder, although no opening from it into the bladder could be found. The interior of the right ureter contained a small amount of granular debris. The uterus was small with only the left tube. This case is one in which another consideration might appear, namely, the division of the mesonephros into a cranial and a caudal part, as shown in Minot's ('10) figures for the pig. The genital gland and the whole duct in Hunt's case and part of the duct in my case were present. In Lyon's case the ovary and the whole tube were missing. These parts are closely associated with the cranial part of the mesonephros. Therefore, my case shows more interference with growth in the region of the caudal mesonephros; Lyon's in the cranial part; while in Hunt's the involvement was caudal, but relatively less. Brown's ('94) case in man shows absence of the right kidney, but the right ureter is present. Harrison ('94) describes in the rabbit an absence of the left kidney and ureter. Both ovaries are present, but uterine tubes and uteri are absent. Here both sides are involved anteriorly. Reterer and Rogers

('93) describe a case in the rabbit with an anomalous condition on the right side similar to the condition described in this paper on the left side (reported by Radasch, '08).

Radasch ('08) gives an extensive review of the literature up to 1908 and concludes that the process causing the loss of kidney is due either to the failure of the ureteric evagination or its retrogression after its appearance. In the case he describes in the cat the presence or absence of the ovary and part of the tube on the right side was not capable of determination, but the right ureter was absent. This would place the condition in a similar class to the one described in this paper.

SUMMARY

A case is described in a rabbit of the absence of the left kidney and ureter, part of the left uterine tube, and the left uterus. By taking into consideration the conditions during embryonic development, the origin of the anomaly is placed at probably from eleven and one-half to fifteen days' embryonic growth. The relationship of this to other reported cases is discussed.

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Resumen por el autor, Warren Lewis.

La cualidad adhesiva de las células.

Es evidente que las células que emigran sobre la superficie inferior del cubreobjetos en los cultivos de tejidos son adhesivas; de otro modo, cuando se emplea un medio de cultivo líquido, caerían al fondo de la gota, puesto que son más pesadas que el medio. Las células son adhesivas a consecuencia del mismo material de que están formadas, del mismo modo que la cola es pegajosa. Sin esta cualidad adhesiva al vidrio y algunos otros sólidos, no podrían emigrar. No existe razón alguna para creer que desarrollan esta cualidad cuando abandonan el trozo cultivado y vienen a ponerse en contacto con el vidrio. Esta cualidad adhesiva varía un tanto, puesto que las células pueden redondearse y desprenderse del cubreobjetos. Este fenómeno es pasivo y no corresponde de modo alguno a la sensibilidad que exhiben ciertos animales para los sólidos, conocida con el nombre de estereotropismo. Las células se adhieren no solo al vidrio y otros sólidos, sino también entre sí en grado variable. Esta cualidad adhesiva juega un papel muy importante en la persistencia del contacto de las células y los diversos tejidos, no solo durante la segmentación y en el embrión antes de formarse las fibras intercelulares, sino también en el adulto. La existencia de continuidad protoplásmica actual, es muy dudosa en la mayor parte de los tejidos, tales como el mesenquima, epitelio y músculo. De tal modo que si las células de nuestro cuerpo perdiesen súbitamente su cualidad adhesiva, nos desintegraríamos repentinamente y los varios tipos celulares formarían una corriente mezclada de ectodermo, músculo, mesenquima, hígado y otros varios tipos celulares.

Translation by José F. Nonidez
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THE ADHESIVE QUALITY OF CELLS

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From observations on the behavior of cells in tissue cultures, it seems to me that the causative factors in the phenomena exhibited by the cells in their migration out from the explant along solid supports resolve themselves into two separate categories: 1) a natural stickiness or adhesiveness which these cells appear to have for certain solids and for each other; 2) the forces that cause them to migrate. Concerning the first very little has been written, although I think we have all been aware that many cells are sticky. We have long known, for example, of the adhesive quality of white blood-cells for glass. The importance of this adhesive quality in the various body cells, however, has been almost entirely ignored. Yet most of our tissues and organs are made up of cells that are merely stuck together. Were these various types of cells to lose their stickiness for one another and for the supporting extracellular white fibers, reticuli, etc., our bodies would at once disintegrate and flow off onto the ground in a mixed stream of ectodermal, muscle, mesenchyme, endothelial, liver, pancreatic, and many other types of cells.

It will seem to many that I am overlooking a very important consideration, namely, the so-called syncytial relations supposed to exist in many tissues, such as the smooth muscle, heart muscle, and especially the mesenchyme, as also the ectoderm, by virtue of its so-called intercellular bridges. I am convinced, after the study of the behavior of these cells in tissue cultures of chick embryos, that the outgrowths of these tissues do not form syncytia. From this it would appear highly probable that they do not form syncytia either in embryos of four to ten days' incubation (the usual ages from which the tissues were taken for cultures) or in the adult, and that this holds true not only for birds,

but for mammals as well. Some of the evidence for the non-syncytial nature of mesenchyme has already been published¹ and that for heart muscle, smooth muscle, and ectoderm is nearly ready for publication.

It is almost self-evident that the cells that migrate out on the under surface of the cover-glass, in cultures where a fluid medium is employed, are sticky for glass. They are often apparently quite firmly adherent, and such preparations can be washed and manipulated in various ways and with various fluids, or even fixed and killed by different agents, without disturbing their attachment. They can even be poked with a needle or centrifugalized, and yet the cells will not be dislodged. Of course, cells do frequently become detached from the glass through manipulations of various sorts, or they may at times round up and drop to the bottom of the hanging-drop, but even after rounding up many still remain attached. Not only the bodies, but the cell processes as well possess this adhesive quality, and it not infrequently happens that an outgrowth, contracting back towards the explant, snaps apart some of the more extended processes at the periphery, leaving slender, isolated fragments of cytoplasm adhering to the cover-glass. The adhesiveness of such fine processes, some of them so thin as to be almost invisible, can scarcely be explained on any other ground than stickiness. It is highly improbable that the adhesion is maintained by some sort of suction apparatus. I do not quite see how it could be proved that the cells are or are not sticky for glass before they come into contact with it, and there is no particular reason for believing that they develop such a special adhesive quality as they leave the explant and come in contact with the cover-slip.

Cells are not only sticky for the cover-glass, but for each other as well. In the body of the embryo and in the tissue explant such cells as endothelium, mesothelium, and the endodermal lining of the intestine are undoubtedly merely adherent to their neighbors, but they retain their adhesive quality in the outgrowths and, in addition, stick to the cover-glass. I believe the same thing is true for those cells that have hitherto been supposed to form syncytia.

¹ Lewis, W. H. Is mesenchyme a syncytium? *Anat. Rec.*, vol. 23, 1922.

We may in time be able to measure the force of the adhesions in some way, as by utilizing the centrifugal force necessary to dislodge them, but so far even the high speed of the ordinary centrifuge fails to move those that are flattened out to any extent from their attachment to the cover-glass. The fact that cells do move and shift about, spread out very flat, or round up and drop off indicates that the factors of cohesion and surface tension of the protoplasm are constantly at work in altering the extent and position of the adherent areas, both on the cover-glass and on the other cells. Such changes may very well take place without altering the natural adhesiveness of the surface of the cells, but it is entirely possible that the degree of stickiness also may vary. One wonders whether this sticky quality is dependent upon the composition of the protoplasmic surface membrane that limits all cells or upon some sort of substance that is secreted by the cell as one of the products of its metabolism. The so-called cement, so commonly described as existing between various types of cells, may possibly be an adhesive substance. This is usually recognized by its power to reduce silver nitrate. Now, most living cells in our cultures show a slight browning over their entire surface with silver nitrate, while dead cells do not; it is therefore possible that the silver nitrate is reduced by the living protoplasm and not by any substance on its surface. The heavy lines seen at the contact edges of cells seem to be, in part at least, an optical effect of looking edgewise at a thin granular film.

The important rôle which this adhesive quality plays in the early stages of development, before the formation of extracellular fibrils of any sort, can scarcely be overestimated. Without it multicellular organisms could not exist. When it is repressed, as was done by Herbst ('00)² in the segmentation stages of certain marine animals, by eliminating calcium salts from the sea-water, the blastomeres fall apart and round up and further development of the embryo becomes impossible. In the blastula and gastrula stages the only other factor that might play a rôle in keeping

² Herbst, Curt. Ueber das Auseinandergehen von Furchungs- und Gewebzellen in kalkfreiem Medium. Arch. f. Entw. Mech., Bd. 9, 1900, S. 424.

the cells in position is the pressure from without of an external membrane inclosing the whole organism. I am eliminating entirely the conception of intercellular bridges, which has been postulated by Hensen³ and others, and have adopted the opposite extreme, which may seem to many as far from the truth as the idea of universal cell continuity. In the early embryonic stages, before the formation of extracellular fibrils and other supporting and binding substances, whether these be in the form of gels or of fibers, all the tissues are held together by adhesion or a combination of adhesion and interlacing of elongated and branched cells. In later stages and in the adult the extracellular supporting substances play an important rôle in holding cells and tissues together, but the intercellular adhesions are probably still more important.

The most obvious deduction to draw from the known facts is that the cells in tissue cultures are sticky as regards certain solids, and that this is a natural characteristic. They are sticky because of the very material of which they are composed, just as egg albumen, glue, and mucilage are sticky. If, then, we admit that the cell surface is naturally sticky for glass, we can scarcely consider that adhesion of the cells to solids is a form of tropism. 'Tropism' as the term is usually employed, applies to the phenomenon observed in living organisms of moving toward (positive) or away from (negative) a focus of light, heat, a solid body (stereotropism), or other stimulus. The term 'stereotropism' has frequently been employed to indicate a reaction exhibited by all of the cells in tissue cultures that migrate out from the explants on solid supports. Harrison ('11, '14)⁴ and others have observed that cells that migrate out from the explant always utilize some solid support, such as the cover-glass, fibrin threads, spider-web, fibers of various sorts, or even the surface film of the fluid hanging-drop. None of the ordinary tissue-cells are able to swim about in fluid media. The idea

³ Hensen, V. Ueber die Entwicklung des Gewebes und der Nerven im Schwanze des Froschlärve. *Virchow's Arch.*, Bd. 31, 1864.

⁴ Harrison, R. G. The reaction of embryonic cells to solid structures. *Jour. Exp. Zool.*, vol. 17, 1914. *Science*, vol. 34, 1911.

underlying the use of the term stereotropism, as applied to the cells in tissue cultures, seems to be that there is some sort of positive reaction on the part of the cells toward the solid. Cells migrate only on solids; therefore it is assumed they are in some way attracted by solids. The following quotation from Harrison ('14, p. 541) indicates the present attitude on the subject:

"While it must therefore be admitted that chemical stimuli may play an important part in influencing the movements of cells in simple cultures, as Burrows has pointed out, the facts show that the cells are also stimulated by solids as such and respond to them by an orienting movement. Response to tactile stimuli is of such general occurrence in animals that there is nothing anomalous in the manifestation of the same kind of sensitiveness in cells."

We say that cells are stereotropic because they have only been observed to migrate on solids, and that they migrate on solids because they are stereotropic. To say that cells are stereotropic does not offer any explanation for the fact that they migrate only on solids. On the other hand, the fact that they can only migrate on solids does not necessarily mean that they are stimulated by solids as such, nor is there any reason to believe in a sensitiveness of cells that corresponds in any way to the response of animals to tactile stimuli. The adhesion of cells to solids is a passive phenomenon. Since cells are heavier than the fluid medium, they could not migrate on the under surface of the cover-slip unless they were adherent.

Concerning the factors which cause the cells to migrate out from the explant along the cover-glass or other solids, on the surface film of a liquid drop, or on other cells (which behave as solids), there is still much uncertainty. The work of Tait ('18)⁵ and Leo Loeb ('20)⁶ indicates that the progressive movements

⁵ Tait, J. The capillary phenomena observed in blood cells, etc. *Quart. J. Exp. Physiol.*, vol. 12, 1918.

⁶ Loeb, Leo. The movements of the amoebocytes and the experimental production of amoebocyte (cell-fibrin) tissue. *Wash. Univ. Studies*, vol. 8, Scientific Series no. 1, 1920.

of the cells may depend upon local variations in the metabolism which produce local changes in the fluidity and surface tension of the cells, while the observations of Burrows⁷ suggest that the interchange of substance between explant and medium may offer a basis for a differential localization of metabolism.

⁷ Burrows, M. T. The tissue culture as physiological method. Trans. Cong. Amer. Physicians and Surgeons, vol. 9, 1913.